Original Research

Hemophagocytic Syndrome: An Experience in a Tertiary Care Center in North India

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ABSTRACT
Introduction: Hemophagocytosis (HPC) is commonly present in cases of infection, malignancy and hemophagocyticlymphohistiocytosis (HLH). But it may also be present in cases of anemia and other benign conditions. High clinical suspicion and early testing is warranted owing to high fatality.
Methods: Retrospective study of cases of HPC reported in the department of Pathology, IMS BHU by studying the previous reports and clinical data available.
Results: The most common condition showing HPC was dimorphic anemia (13.3%) followed by infective etiology (6.7%). HLH was diagnosed in 8.9% of the cases with a mean age of 50 years and a male preponderance.
Conclusion: Hemophagocytosis is often overlooked in bone marrow examination and not documented. Even a single instance must be reported as it may be the only sign of infection.
Keywords: Hemophagocytosis, HLH, Infection, Malignancy.

INTRODUCTION

Hemophagocytosis maybe defined as the engulfing of blood and its precursors by macrophages or histiocytes that may lead to cytopenia.[1] HPC is often overlooked inBM examination and not documented many a time in the reports, however when suspecting infection, it may be the only sign and extensive search for causative organisms like tuberculosis, leishmaniasis, fungal elements and malarial parasites must be carried out.HPC must be differentiated from emperipolesis wherein the cells can exit the cytoplasm without any morphological or functional injury. Macrophage activation syndrome is associated with autoimmune diseases such as Still's disease and Systemic Lupus Erythematosus (SLE).[2]Hemophagocyticlymphohistiocytosis (HLH) is a clinical syndrome of immune regulation characterized by continuous high-grade fever, cytopenias, hepatosplenomegaly due to a storm of cytokines released from dysregulated activation of macrophages, natural killer cells (NK cells) and T-cells leading to systemic inflammation and multi-organ dysfunction contributing to 75% of deaths in the intensive care unit.[3-5] Based on the etiology, HLH maybe hereditary and acquired. *PRF1*, *UNC13D*, *STX11* and *STXBP*, coding for Perforin, Munc13-4, Syntaxin11, and Syntaxin binding protein 2 respectively have been implicated in familial HLH.[3,6]With an incidence of 1.2/million children/year and mortality rate ranging from 20 to 30% within 2 months of diagnosing familial HLH, identifying pathogenic biallelic variants in carriers and early hematopoietic stem cell transplantation improves prognosis.[7,8]High turn-around time of sequencing forces clinicians to rely on clinical and laboratory features due to the time-ticking mortality rate. Familial and acquired HLH maybe differentiated

by obtaining a detailed family history.[9]HPC can be classified into negative (no HPC) mild (< 2 occurrences of HPC/smear), moderate (2 to 5 occurrences of HPC/smear) and severe (> 5 instances of HPC/smear).[10]HPC in BM, preferred due to easy accessibility, is neither sensitive nor specific with presence ranging from 25 to 100% in HLH.[11,12]Flow cytometric analysis of low Natural killer cell activity and perforin are positive in only half of the cases making HPC in the bone marrow (BM), spleen, lymph node and liver, significant.[11,13]Serum soluble IL-2 and ferritin have a sensitivity of 0.93 and 0.84.[1] However, these tests are limited in availability. Secondary HLH (sHLH), seen in adults with infections among other triggers like autoimmune disease and malignancies has a mortality rate varying from 30 to 40% within the first two months of diagnosis and around 80% in the intensive care unit.[5,8,14]The common infections causing sHLH in India are Tuberculosis, kala-azar and enteric fever.[14,15] A systematic review based on 65 studies including 661 patients showed the triggers of sHLH to be infections, malignancies, autoimmune diseases, unidentifiable causes and drugs with incidences of 49.9%, 28%, 12.1%, 9.4% and 0.6% respectively.[5]Few studies on critically ill patients have found a prevalence of HLH to be 43% on screening patients with fever and bicytopenia, but these studies have acknowledged the missing of cases due to COVID-19 and Epstein-Barr virus infections.[14,15] Treatment is based on identifying the trigger and the use of immunosuppressive agents like steroids, cyclosporine and etoposide[5,9,15] and is undertaken even if criteria for HLH is not fulfilled in the setting of high clinical suspicion.[9]

MATERIALS AND METHODS

This was a retrospective study conducted in the department of Pathology, Institute Medical Sciences, Banaras Hindu University (Tertiary care centre of Uttar Pradesh). In this study we have taken cases diagnosed with HLH or showing HPC over a period of 2 years from 2021 to 2023. The study was conducted after obtaining ethical clearance from the Institutional ethics committee. Clinical records were reviewed,detailed history, presentation, laboratory data and BM aspirate examination findings noted. Diagnosis of HLH was based on the 2004 guidelines that requires either the presence of a mutation in a gene known to cause familial HLH or five out of eight criteria composed of clinical, laboratory, flowcytometric and histological features. The clinical criteria comprised fever >38.5ºC, lasting for more than 7 days and splenomegaly, spleen palpable at least 3cm below the left costal margin. The laboratory criteria comprised cytopenias of at least two lineages in the peripheral blood (hemoglobin < 9 g/dl, platelets < 100,000/cu mm or absolute neutrophil count < 1000/cu mm), hypertriglyceridemia or hypofibrinogenemia (fasting triglycerisdes> 177mg/dl or 3 standard deviations (SD) more than normal value for age, fibrinogen < 150mg/dl or > 3SD less than normal value for age), serum ferritin > 500ng/ml and increased soluble interleukin-2 (CD25) levels (>2400 U/ml or very high for age). Documentation of low or absent NK cell activity on flow cytometry.[9]A minimum of three smears and 500 nucleated cells were examined for each case. BM aspiration was followed by BM biopsy for confirmation of diagnosis and underlying cause. Special stains were used wherever necessary.Descriptive statistics were summarized in the form of mean, median and standard deviation and percentage.

RESULTS

There was a total of 45 cases showing HPC on BM. HLH was diagnosed in 4/45cases (8.8%) that fulfilled the HLH criteria. The cases that showed HPC and did not fulfill HLH criteria were taken as Hemophagocytic syndrome due to other etiology, seen in 41/45 cases (91.2%). The demographics have been shown in Table 1. The mean age of presentation in HLH was 50 years as compared to 37 yearsin non-HLH patients and a male predominance was noted. Pancytopenia was the most common finding on peripheral blood examinationand formed the most common indication for BM aspiration for ruling out HLH. Hypercellularity was observed in 16% of all the cases. Infective etiology, dimorphic anemia and HLH formed the most common BM examination impressions.BM biopsy was performed in 1 case of HLH and was hypocellular.Reticulin stain did not highlight fibrosis in any of the cases. Perl's stain showed low iron stores in 3 cases.

Table 1: Demographics

Parameter	Total (45 cases)	HLH (4 cases)	HPC cases (41 cases)
Age (mean ± SD in years, range)	37.28±21.96 (2 – 87)	50±16.87(28-75)	36.05±22 (2-87)
Gender (number of males, male:female ratio)	29, 1.8	3,3	26,1.7

Table 2: Summary of clinical presentation

Parameter	Total (45 cases)	HLH (4 cases)	HPC cases (41 cases)
Lymphadenopathy	6(13.3%)	2(50%)	4(9.8%)
Splenomegaly	19(42.2%)	4(100%)	15(36.6%)

Hepatomegaly	14(31.1%)	4(100%)	10(24.4%)
No organomegaly and lymhadenopathy	6(13.3%)	0	6(14.6%)
Fever	34(75.6%)	4(100%)	30(73.1%)
Increased serum ferritin level (normal: 12 to 300ng/mL in males, 12 to 150 ng/mL in females)	Increased in 34 cases (75.6%)	Increased in 4 cases (100%)	Increased in 30 cases (73.1%)

Table 3: Summary of laboratory parameters

Parameter	Total	HLH cases	HPC cases
Hemoglobin (g/dl) (mean (range))	7.92 (4.2 – 14.9)	8.15 (4.3-14.9)	7.89 (4.2 – 13)
RBC morphology **Normocytic normochromic (NCNC)** **Anisopoikilocytosis (ANPK)** **Microcytic hypochromic (MCHC)** **Microcytic normochromic (MCNC)**	ANPK – 21 (53.8%) NCNC – 10 (25.6%) MCHC – 5 (12.8%) MCNC – 1 (2.5%) Macrocytic – 1 (2.5%) Poorly preserved – 1 (2.5%)	ANPK – 3 (75%) Poorly preserved – 1 (25%)	ANPK – 18 (51.4%) NCNC – 10 (28.6%) MCHC – 5 (14.3%) MCNC – 1 (2.9%) Macrocytic – 1 (2.9%)
Absolute neutrophil count (ANC) (/cu mm)	3440 (33 – 13000)	733(33-1620)	3405(154-13270)
Platelet count (mean((range)) in /cu mm	108889 (243 - 520000)	42500(1000-139000)	117714(243-524000)
Hemoglobin < 9g/dl	24(68.5%)	3 (75%)	21(67.7%)
Platelet <1 lakh/ cu mm)	20(57.1%)	3 (75%)	17(54.8%)
ANC (<1000/ cu mm)	16(45.7%)	3 (75%)	13(41.9%)
Leukocytosis (>11000/cu mm)	4 (11.4%)	1 (25%)	3 (9.7%)
Pancytopenia	5(14.3%)	1(25%)	4 (12.9%)
Bicytopenia (RBC and platelet lineage)	9(25.7%)	1 (25%)	8 (25.8%)
Bicytopenia (RBC and WBC lineage)	5(14.3%)	0	5 (16.1%)
Bicytopenia (platelet and WBC lineage)	3(8.6%)	1 (25%)	2 (6.5%)

Table 4: Spectrum of clinical presentation in suspicion of HLH

Indication for BM examination	Number of cases
Pancytopenia for evaluation	5
Hodgkin's lymphoma	2
Acute lymphoblastic leukemia	2
Acute myeloid leukemia	1
Autoimmune hemolytic anemia	1
Anemia under evaluation	1
Known case of HLH	1

Table 5: BM examination findings.

Parameter	Total (45)	HLH cases (4)
BM aspirate celluarity	16 (35.5%) - Hypercellular 12 (26.7%) – Normocellular 4 (8.8%)– Hypocellular 4 (8.8%)– Normocellular to hypocellular 4 (8.8%)– Normocellular to hypercellular 3 (6.6%) – Aparticulate 2 (4.4%) – Variably cellular	2 (50%) – Normocellular 2 (50%) – Hypercellular
BM biopsy (done in 9 cases)	5 – Normocellular 2 – Hypocellular 1 - Hypercellular	1 - Hypocellular

Table 6: Summary of BM examination findings.

Diagnosis	Number of cases (Total = 45)
HLH	4 (8.9%)

Hemophagocytic syndrome (other etiology)	27 (60%)
Dimorphic anemia	6 (13.3%)
Infective etiology	3 (6.7%)
Micronormoblastic anemia	2 (4.4%)
Megaloblastic anemia	2 (4.4%)
Iron deficiency anemia	1 (2.2%)

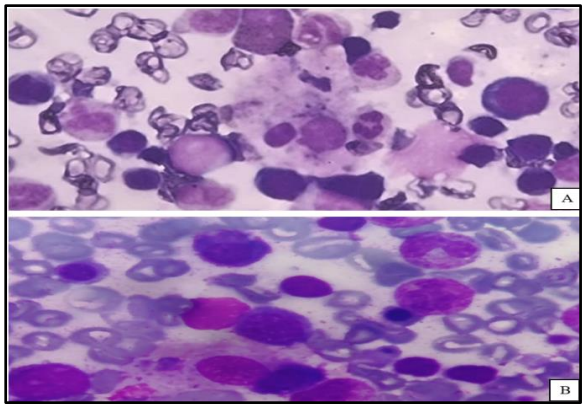

Figure 1: HPC seen in various cases (Leishman stain) A. Dimorphic anemia, B. Infection

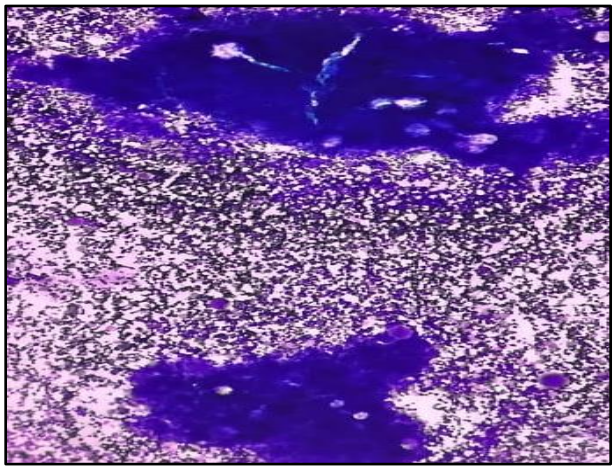

Figure 2:Bone marrow smear showing hypercellularmarrow in a case of HLH (Leishman stain)

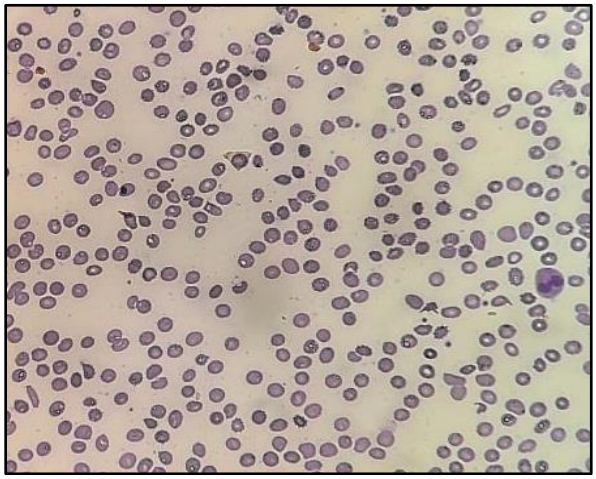

Figure 3: Peripheral blood smear showing pancytopenia (Leishman stain)

DISCUSSION

HLH presents a challenge to clinicians due to variable clinical presentations. The HLH criteria were primarily defined for enrollment into clinical trials.[16]There is no approved gold standard diagnostic test. In a study conducted in the Himalayan region, Chandra et *al.* has found infections and HLH to be most common cause. In our study, pancytopenia was seen in 14.3 % of the cases.Similar to our study, pancytopenia was the predominant finding on BM examination.[17]In a study by Gars et *al.* on 40 HLH patients, 56% of them had an underlying malignancy and 32.5% of the cases showed evidence of Epstein Barr virus infection. They found that 73% of patients met the HLH criteria irrespective of the presence of HPC whereas HPC was an important criterion required for diagnosis in 22% of the patients. In our study, the most common condition showing HPC was dimorphic anemia (13.3%) followed by infective etiology (6.7%).Noting the cell-lineage of phagocytosis was important as granulocyte ingestion along with presence of multinucleated cells within the cytoplasm a single histiocyte has a significant association with HLH.[18] HPC has been found in up to 4% patients with cytopenias and sepsis without HLH based on a study in critically ill patients.[19]Fazal et *al.* obtained a sensitivity of 90.9% with respect to BM scoring in HLH diagnosis, higher than H-score and serum ferritin levels, however, BM score specificity of 37.2% was lower than H-score and ferritin levels.In their study, threshold hemoglobin value of 13g/dl and 12g/dl has been taken to define cytopenias. Infection was the most common trigger, similar to results obtained from a case series by Kumar et *al.*who found viral causes predominantly Ebstein-Barr virus, cytomegalovirus and parvovirus followed by bacterial causes, leishmaniasis and fungal etiology.[15,20]Pannu et *al.* found thrombocytopenia to be the most common hematological finding whereas in our study, anemia was the most common finding.A 5-year study in South India by Bhatti et *al*, has also found infection to be the most common cause of HPC (18%) and hypercellularity as the predominant BM finding, similar to our study. They obtained a significant correlation between thrombocytopenia and HPC grade. Studies regarding the correlation between grade of HPC and severity of disease have shown inconsistent results.[21]The underlying etiology could not be established in all cases in most of these studies as in our study which was taken as hemophagocytic syndrome of unknown etiology (60% cases).A retrospective study on 10 patients with median age of 25.5 years and bicytopenia in 40% of cases by Rajagopala et *al.*shows the rapid course of HLH by highlighting the high mortality rate with shock and acute respiratory distress syndrome being the common causes of death.[16]Deterioration of the patient's condition may make the patient eligible for HLH criteria after sometime, therefore high clinical suspicion is paramount. Specific treatments focused on the HLH pathway pathobiology are lacking owing to the heterogeneity leading to difficulties designing randomized controlled trials.

CONCLUSION

A collaborative approach between clinicians and laboratory consultants is necessary to make calls in this rapidly fatal syndrome as patients may progress to develop all the features of HLH but with less time remaining. More studies are required from India regarding the spectrum of clinical presentations, laboratory features and BM findings to help differentiation from prevalent tropical infections and sepsis.When not responding to therapy, bone marrow examination must be done to look for HPC. In absence of fulfilling HLH criteria, HPC in the bone marrow should not be overlooked. These cases need special attention in terms of investigation and treatment.

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